

Monoclonal Anti-*Mycobacterium tuberculosis* LpqH (Gene Rv3763), Clone B (8E6-A5) (produced *in vitro*)**Catalog No. NR-50098**

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Product Description:

Antibody Class: IgM

Monoclonal antibody to *Mycobacterium tuberculosis*, strain H37Rv 19 kDa lipoprotein (LpqH; Rv3763), clone B (8E6-A5) was produced in cell culture using a B cell hybridoma generated by the fusion of myeloma cells with immunized mouse splenocytes.

Lot: 70006605**Manufacturing Date: 27NOV2018**

Production and QC testing were performed by Colorado State University (CSU). The CSU documentation for lot 18.anti-19kDa.B.8.3.19.mm is attached.

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Work Sheet for Antibodies

General Information:

BEI Catalog Number: NR-50098
 Product Description: Anti-19kDa hybridoma (LpqH, Rv3763), Clone B (8E6-A5)
 CSU Lot Number: 18.anti-19kDa.B.8.3.19.mm
 Species: *Mycobacterium tuberculosis*
 Strain: H37Rv
 Type (select one): Mouse Monoclonal
 Rabbit Polyclonal
 Guinea Pig Polyclonal

Production Information:

Cell Line: 18.anti-19kDa.B.8.16.19.Hyb SOP#: AB103.5, AB104.4 Notebook/pp: Monoclonal Antibody #3 BEI, pg. 47-113
 Amount of CS Harvested: ~25.4 mL Clarity: clear, filtered after ammonium sulfate precipitation dialysis
 IgG Purification: N/A SOP#: N/A Notebook/pp: N/A
 Antibody Precipitation: 50% ammonium sulfate precipitation¹, followed by dialysis into 1X PBS, final volume of ~7mL after dialysis (some used for QC)
 Notebook/pp: Monoclonal Antibodies #3 BEI, pg. 115-last pages and on ammonium sulfate precipitation protocol attached in notebook, Monoclonal Antibodies #4 BEI, pg. 6-12, 17-19
 Ig isotype: IgM SOP#: AB106 Notebook/pp: Monoclonal Antibodies #4 BEI, pg. 25

QC Information:

Tested Against: 1µg ova conjugated peptide H2N-IAIGGAATGIAAVLTDGNPP(dPEG4)CKKK-amide, 5µg TX-114
 * TX114 is a fraction enriched with lipoproteins such as Psts1 (38kDa, Rv0934c), LprG (25kDa, Rv1411c), and LpqH (19kDa, Rv3763c). (Wolfe et al, 2010. JPR)
 SOP#: AB102.1, SPO39.1 Notebook/pp: Monoclonal Antibodies #4 BEI, pg. 18-19
 Tested by: Western blot: Titer: 1: 1000
 ELISA: Titer: 1: 100
 Special Instructions: 3% BSA recommended for blocking. Long substrate exposure time (>10 minutes) may be necessary for developing western blots.

QC ELISA:

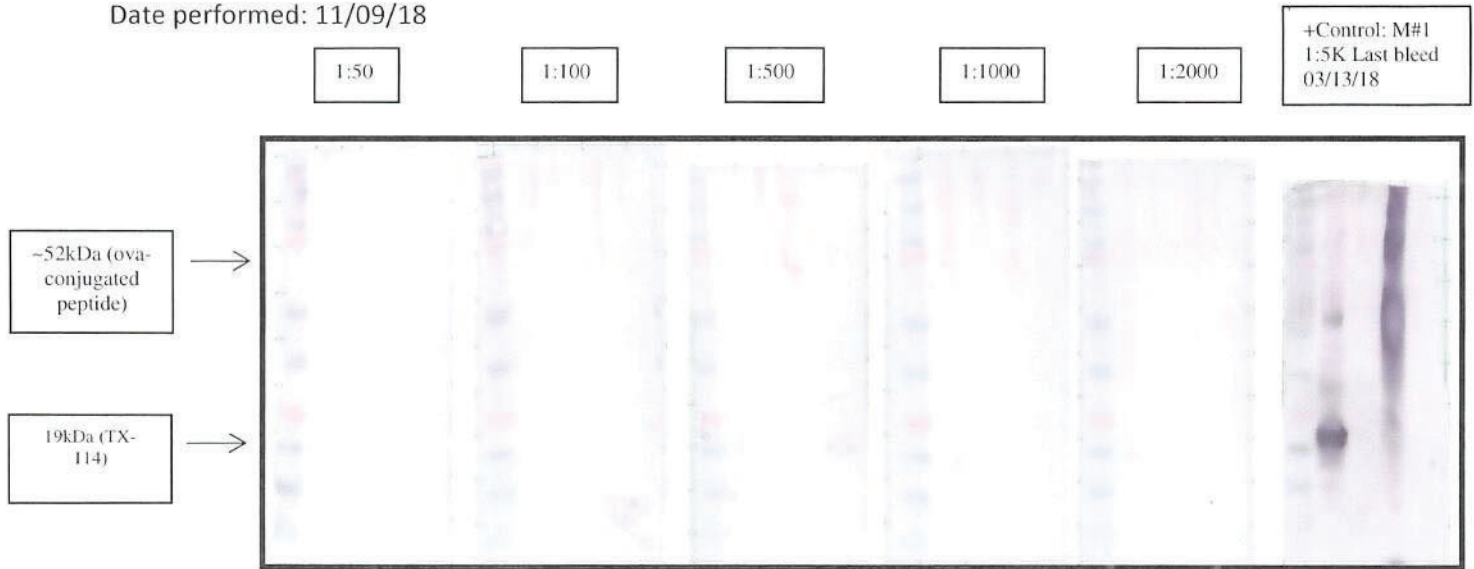
Date performed: 11/09/18

Titer	30 minutes, 1µg conjugated ova-peptide (averages)	45 minutes, 1µg conjugated ova-peptide (averages)	30 minutes, 5µg TX-114 (averages)	45 minutes, 5µg TX-114 (averages)	Date
1:50	1.364	1.962	0.105	0.12	11/09/18
1:100	1.026	1.476	0.123	0.135	11/09/18
1:500	0.427	0.602	0.0875	0.0945	11/09/18
1:1000	0.325	0.442	0.089	0.0945	11/09/18
1:2000	0.203	0.266	0.0915	0.0975	11/09/18
Positive Control (1:5K Mouse#1 Polyclonal Antibody, Last bleed 3/13/18)	OVERFLOW	OVERFLOW	0.696	0.994	11/09/18
Negative Control (TBST)	0.0785	0.0795	0.089	0.0945	11/09/18

Note: Antibody reactive against linear peptide. Therefore, if running an ELISA for native protein, a denatured ELISA protocol should be used².

QC Western blot:

Date performed: 11/09/18



Gel Order: M-5µg Tx-114-blank-1µg ova-conjugated peptide-blank

Aliquot Information: 40 x 100µL aliquots (BEI), 7.5 x 100µL aliquots (In-House, QC)

Kala Early 11/27/18
(Research Associate) (date)

C. Mahaffey 11/27/18
(Laboratory Supervisor) (date)

References:

1. Grodzki A.C., Berenstein E. (2010) Antibody Purification: Ammonium Sulfate Fractionation or Gel Filtration. In: Oliver C., Jamur M. (eds) Immunocytochemical Methods and Protocols. Methods in Molecular Biology (Methods and Protocols), vol 588. Humana Press
2. Hnasko, R., Lin, A., McGarvey, J.A., & Stanker, L.H. (2011). A rapid method to improve protein detection by indirect ELISA. *Biochemical and Biophysical Research Communications*, 410, Issue 4, 726-731.